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EXAMINER	
FOSTER, CHRISTINE E	

ART UNIT	PAPER NUMBER
1641	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/620,332	VOYTA ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 November 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19 and 21-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-19 and 21-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

Applicant's amendments of claims 1, 3, 5, 8, 13, 17-18, 21, 22-24, 26, 30-31, and 35-38 in the reply filed 11/1/05 are acknowledged and have been entered.

Regarding the amendment of claim 1, Applicant is reminded that the text of each claim under examination must be with markings to show current changes (see MPEP 714). The word "conjugate" which appears in the last line of claim 1 as originally filed, is not present in the amended claim.

Applicant's cancellation of claim 20 is acknowledged and has been entered (see Applicant's response, p. 19). Applicant is reminded that the claim status should be indicated in a parenthetical expression following the claim number, i.e. as "(canceled)".

Claims 1-19 and 21-45 are currently pending.

Specification

The objections to the specification are withdrawn in response to Applicant's amendments.

Claim Objections

The objections to claim 5 are withdrawn in response to Applicant's amendments.

Claim Rejections - 35 USC § 112

1. Claims 1-19 and 21-45 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Amended claim 1 recites that "at least some of the probes are bound to a first enzyme conjugate comprising the first enzyme prior to contacting the surface layer of the solid support with the composition comprising the first chemiluminescent substrate, and wherein at least some of the probes are bound to a second enzyme conjugate comprising the second enzyme [conjugate] prior to contacting the surface layer with the composition comprising the second chemiluminescent substrate". Support cannot be found for the new limitations (underlined), and Applicant did not indicate where such support may be found in the specification.

Amended claims 24 and 30 recite that fluorescent labels are attached directly or indirectly to the surface layer. The specification discloses that the support surface may comprise fluorescent labels (p. 9, lines 11-18), but support cannot be found for fluorescent labels attached to the surface layer, and Applicant has not indicated where such support may be found in the specification.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 10-12 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting chemiluminescent emissions on a solid support wherein probes are disposed on the surface layer at a density of at least 50 or at least 100 discrete areas per cm^2, does not reasonably provide enablement for a method wherein the density is at least 1,000, 25,000 or 50,000 discrete areas per cm^2. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are directed to a method of detecting binding of target species to an array of probes immobilized on a solid support. Binding of targets accompanied by co-immobilization of enzymes capable of cleaving chemiluminescent substrates, which allows for subsequent detection of chemiluminescent emissions when the solid support is contacted with the substrates. A large number of different probes may be immobilized on the support, thereby allowing for screening for the presence of multiple targets in a single assay. The claims are not limited to arrays produced by any particular method and are not limited to a type of probe biomolecule that is immobilized on the support.

Arrays of biomolecules immobilized on solid supports are known in the art and include arrays of proteins (typically known as "protein arrays" or "protein biochips") and nucleic acids ("DNA microarrays" or "cDNA microarrays"). The prior art teaches methods of preparing DNA microrarrays that include ink-jet systems, direct or robotic-aided printing/spotting, and photolithography (Hughes et al., "Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer," *Nature Biotechnology* **19**:342-347 (2001); Reese et al., "Microfabricated Fountain Pens for High-Density DNA Arrays," *Genome Research* **13**:2348-

2352 (2003)). Photolithography was successful in creating DNA arrays with approximately 17,000 genes/cm^2 (Reese et al., p. 2348, left column, paragraph 2). Printing using ceramic capillary-tipped pens produced arrays with 10,000 spots/cm^2 (Reese et al., p. 2348, right column, paragraph 1). However, arrays with feature densities of greater than 25,000 were not commonly known and available at the time of the invention. Moreover, protein-based microarrays were not well known in the art (Joos et al., p. 2641, right column, lines 13-15) and did not approach the feature densities of DNA-based microarrays, with less than 1,000 features (see Mendoza et al., "High-Throughput Microarray-Based Enzyme-Linked Immunosorbent Assay (ELISA)" *BioTechniques* 27:778-788 (1999)).

The prior art teaches detection strategies for use in conjunction with arrays, which include fluorescence and chemiluminescence, with fluorescence detection being most generally used for DNA microarrays (Rajeevan et al., "Chemiluminescent Analysis of Gene Expression on High-density Filter Arrays," *The Journal of Histochemistry* **47**:337-342 (1999), p. 337, left column). The prior art does teach chemiluminescent detection using DNA arrays (e.g., Rajeevan et al; Vernon et al., "Reproducibility of Alternative Probe Synthesis Approaches for Gene expression Profiling with Arrays," *Journal of Molecular Diagnostics* **2**:124-127 (2000); Cheek et al., "Chemiluminescence Detection for Hybridization Assays on the Flow-Thru Chip, a Three-Dimensional Microchannel Biochip," *Anal. Chem.* **73**:5777-5783 (2001)) as well as protein arrays (e.g., Roda et al., "Protein Microdeposition Using a Conventional Ink-Jet Printer," *BioTechniques* **28**:492-496 (2000) (Joos et al., "A microarray enzyme-linked immunosorbent assay for autoimmune diagnostics," *Electrophoresis* **21**:2641-2650 (2000)).

However, in contrast to the feature densities listed above, these prior art teachings of chemiluminescent detection methods employ arrays of more modest feature densities. Cheek et al. teach 8x8 arrays of probes spotted on 1-cm^2 chips, while the filter arrays of Rajeevan et al. and Vernon et al. comprised 588 spotted targets. Roda et al. teach ink-jet printing of 100-member arrays on a 20 x 20 mm support, with a minimum (Figure 1 and p. 496, middle column). Joos et al. achieved densities on the order of 100 spots per cm^2 (see Joos et al., p. 2643, section 2.2).

Therefore, chemiluminescent detection methods using arrays with densities on the order of 50-100 discrete areas per cm^2 were taught by the prior art at the time of the invention. However, chemiluminescent detection of high-density arrays as claimed in claims 10-12 were not well known in the art, and the specification provides no working examples of any array density.

Regarding production of the probe arrays on the solid support, the specification discloses only that "a plurality of probes are disposed in a plurality of discrete areas on the surface layer" (p. 6) but does not describe or suggest methods of producing high-density arrays or detail how the probes are immobilized on the surface layer according to the instant invention. A number of possible support materials are disclosed, including nitrocellulose, glass, and plastic, but there is no guidance provided regarding how to immobilize probes on the support or how to produce high-density arrays of the claimed densities on these materials. The claims encompass nucleic acid, protein, and other probe types, but the specification further lacks guidance with regard to production of high-density arrays and probe immobilization when the various probe types are to be used according to the invention.

The prior art also teaches that the quality of chemiluminescent signal detection from solid supports is influenced by the type of support used and/or the type of probe immobilized on the support. Roda et al. teach that conventional cellulose paper was a poor support as it gave a poorly localized signal (p. 494, left, column, paragraph 3). Akhavan-Tafti et al. teach that different supports led to substantial variability in apparent spot size, which would affect spatial resolution, and further that certain supports such as glass slides were unsuitable for immobilization of DNA (Akhavan-Tafti et al., "Chemiluminescent Detection of DNA in Low- and Medium-Density Arrays," *Clinical Chemistry* **44**:2065-2066 (1998), p. 2066, right column).

Technical barriers that may be present with chemiluminescent detection and not with fluorescent detection of microarrays include the requirement of enzymes in chemiluminescence detection: Roda et al. teach that chemiluminescent signals may be poorly localized due to washing away of the chemiluminescent enzyme (p. 494, left column, paragraph 3). The specification also discloses that "in contrast to fluorophore-labeled targets, the use of enzyme labeled targets and chemiluminescent substrates results in a signaling species...which is not attached to the support and which is therefore free to migrate during the assay...[which]...can reduce the spatial resolution of the assay (see p. 2, lines 10-20). The specification discloses flash kinetics (p. 4, lines 18-23), which is also taught by Cheek et al. to address the problem of spatial resolution (p. 5779, left column, second paragraph). However, the specification lacks guidance regarding how to use the methods of the invention with the substantially higher density microarrays claimed in claims 10-12.

Due to the state of the prior art, which establishes that microarrays of the claimed densities, and in particular chemiluminescence detection in conjunction with such microarrays,

were not well known in the art at the time of the invention, the lack of direction/guidance presented in the specification regarding production of such high-density arrays and chemiluminescence detection methods employing them, the absence of working examples directed to same, the unpredictability of adequate signal detection using various support materials, and the breadth of the claims, which encompass both protein, DNA, and other arrays produced by any method, the specification fails to teach the skilled artisan how to make and use the claimed invention without undue experimentation.

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-19 and 21-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

5. Claim 1 is rejected as vague and indefinite for recitation of probes, "wherein at least some of the probes are bound to a first enzyme conjugate...and at least some of the probes are bound to a second enzyme conjugate." It is unclear whether the probes are bound to the enzyme conjugates throughout the method (e.g., covalently bound), or whether the probes become bound to the enzyme conjugates at some point in time during the performance of one of the method steps. Applicant has amended the claim to recite that the probes are bound to the enzyme conjugates prior to contacting the surface layer of the solid support with the substrate compositions (see Applicant's amendment, p. 4-5). However, it is still unclear whether the binding of the probes to the enzyme conjugates is an active process step, i.e., the enzyme

conjugates are added to the solid support such that they interact with and bind to the probes, or whether the enzyme conjugates are attached to the probes, such as during the manufacture of the microarray, and remain bound throughout the method.

6. Claim 1 recites the limitation "the second enzyme" (see the amended claim set, p. 4, the last line). There is insufficient antecedent basis for this limitation. The word "conjugate" appears to have been inadvertently deleted from the claim.

7. Claims 3-4 and 7 recite methods according to claim 1, wherein enzyme conjugates are "bound to" a probe. Claim 4 additionally recites target molecules that are "bound to" probes. The claims are indefinite because it is unclear whether the enzyme conjugates and target molecules are bound to the probes throughout the method (e.g., covalently bound), or whether the enzyme conjugates and target molecules become bound to the enzyme conjugates at some point in time during the performance of one of the method steps. As discussed above, the use of the present tense renders it unclear whether the binding is an active method step or a description of the structure of the solid support.

8. Claim 31 recites "a sample comprising the first target molecule". It is unclear whether this sample is the "sample comprising first target molecules...and second target molecules" as recited in claim 13, or a distinct sample.

Claim Rejections - 35 USC § 102

9. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. Claims 1, 3-4, 13-15, 21, 29, and 31-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Cheek et al. ("Chemiluminescence Detection for Hybridization Assays on the Flow-Thru Chip, a Three-Dimensional Microchannel Biochip," *Anal. Chem.* **73**:5777-5783 (2001)).

Cheek et al. teach a method of sequentially detecting chemiluminescent emissions on a solid support (microchannel glass) that includes the steps of contacting a surface layer of the solid support with a composition comprising a first chemiluminescent substrate (the luminol-based substrate Super Signal West Femto Maximum Sensitivity Substrate) capable of being activated by a first enzyme (horseradish peroxidase) to produce a first chemiluminescent signal; detecting the first chemiluminescent signal (see p. 5780, left column, "Detection," and pp. 5781-5782, "Two-Channel Chemiluminescence"); contacting the surface layer of the solid support with a composition comprising a second chemiluminescent substrate (the acridan phosphate-based substrate APS-5) capable of being activated by a second enzyme (alkaline phosphatase) to produce a second chemiluminescent signal; detecting the second chemiluminescent signal; wherein a plurality of probes are disposed in a plurality of discrete areas on the surface layer at a density of 64 discrete areas per cm^2 (see p. 5778, lines 2-14; p. 5779, left column, "Chip Preparation," lines 1-4 and right column, lines 11-12).

The first and second enzyme conjugates are each bound indirectly to a probe (biotin-labeled target molecules are bound to streptavidin-HRP conjugates via biotin-streptavidin interaction and FITC-labeled targets are bound to anti-fluorescein-alkaline phosphatase conjugates via FITC/anti-fluorescein interaction). The labeled nucleic acids are contacted with

the support surface prior to addition of the substrate composition (see Table 1 and pp. 5781-5782, "Two-Channel Chemiluminescence"). Detection and quantification of target molecule binding to discrete areas of the chip is through image analysis (p. 5782, left column and Figure 5). The solid support is washed before contact with the first and second substrate compositions (p. 5779, "Hybridization assay," and p. 5782, left column). The enzyme conjugates were bound to the probes prior to contacting the surface layer of the solid support with the compositions comprising the chemiluminescent substrates (see p. 5781-5782, "Two-Channel Chemiluminescence" and p. 5779-5780, "Hybridization Assay" and "Detection").

11. Claims 25 and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Cheek et al. as evidenced by Weimer et al. (US Patent Application Publication 2004/0009529 A1). Cheek et al. is as discussed above, which fails to specifically recite that the luminol and acridan phosphate-based substrates employed have the same or different emission maxima.

Weimer et al. teach that luminol emits light at a maximum of 425 nm, and that APS-5 emits light at a maximum of 430 nm (paragraph 66). Therefore, the luminol and acridan phosphate-based substrates of Cheek et al. inherently anticipate claim 25, as they have different emission maxima. With respect to claim 27, because the instant specification and claims do not define what wavelengths or range of wavelengths constitute "approximately the same" emission maxima, the examiner has considered that the emission maxima of 425 and 430 nm could fulfill this limitation as well, thereby anticipating instant claim 27.

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

14. Claims 2, 5, 28, and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. (*Anal. Chem.* **73**:5777-5783 (2001)) in view of Akhavan-Tafti (US Patent No. 6,068,979).

Cheek et al. is as discussed above, which fails to fails to specifically recite that the chemiluminescent substrate compositions are contacted with the support in the presence of a quantum yield enhancing material. With regard to claim 44, Cheek et al. fails to teach antidigoxigenin:enzyme conjugates wherein the corresponding target molecules are labeled with digoxigenin. Cheek et al. also fail to specifically recite that the compositions comprising the chemiluminescent substrates are buffered compositions.

Akhavan-Tafti '979 teaches a method for sequential detection of multiple analytes by chemiluminescent emission on a solid support (the abstract), wherein surfactant enhancers are used to improve the signal/background ratio of enzymatically produced chemiluminescence. Akhavan-Tafti '979 further teaches that suitable surfactant enhancers are known in the art and include polymeric onium salts, including quaternary phosphonium salts and ammonium salts, monomeric quaternary phosphonium and ammonium salts such as cetyltrimethylammonium bromide and dicationic surfactants (column 10, lines 29-47).

Akhavan-Tafti '979 teaches two chemiluminescent substrates that are employed in the method and are used in buffered compositions (column 10, lines 42-47 and column 15, lines 1-17). Akhavan-Tafti further teach that the dioxetane chemiluminescent substrate LUMIGEN PPD is commercially available as the composition LUMI-PHOS PLUS, which comprises an alkaline buffer solution.

Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Therefore, it would have been obvious to one of ordinary skill in the art to employ surfactant enhancers, as taught by Akhavan-Tafti '979, in order to improve the chemiluminescent signal/background ratio in a method for sequential detection of enzymatically produced chemiluminescence, such as that of Cheek et al. It would also have been obvious to one of

ordinary skill in the art to employ buffered compositions comprising chemiluminescent substrates in the method of Cheek et al. because Akhavan-Tafti teaches that such compositions are commercially available in that form and are useful in a method for sequential detection of chemiluminescent emissions on a solid support, such as that of Cheek et al.

It would also have been obvious to one of ordinary skill in the art at the time of the invention to substitute the streptavidin-HRP conjugate of Cheek et al. with an antidigoxigenin-HRP conjugate for detection of a second target molecule labeled with digoxigenin, because Akhavan-Tafti '979 teaches that antigen-antibody and biotin-streptavidin are both examples of binding pairs that may be successfully used in a method of sequential chemiluminescent detection of multiple, differentially labeled target molecules such as that of Cheek et al.

15. Claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. ("Chemiluminescent Detection of DNA in Low- and Medium-Density Arrays," *Clinical Chemistry* **44**:2065-2066 (1998)).

Akhavan-Tafti '979 teaches a method of detecting chemiluminescent emissions on a solid support substantially as claimed. The method includes the steps of contacting a surface layer of the solid support with a composition comprising a first chemiluminescent substrate capable of being activated by a first enzyme to produce a first chemiluminescent signal, detecting the first chemiluminescent signal on the surface layer of the solid support, contacting the surface layer of the solid support with a composition comprising a second chemiluminescent substrate capable of being activated by a second enzyme to produce a second chemiluminescent signal, and detecting the second chemiluminescent signal on the surface layer of the solid support (see in particular

column 6, lines 17-24, 41-45 and 60-67; and column 7, lines 1-46; column 10, lines 59-67; and column 11, lines 1-44).

Akhavan-Tafti '979 also discloses use of chemiluminescent quantum yield enhancing materials, which may be onium copolymers (column 10, lines 24-47) including poly(vinylbenzylammonium salts) and which may be present in the chemiluminescent substrate composition (see column 9, lines 46-52; column 10, lines 15-47 and US Patent 5,45,347, which was incorporated by reference). Chemiluminescent substrates are employed as buffered compositions (column 9, lines 46-47; column 10, lines 42-45, and column 15, lines 1-8). Washing of the solid support may be performed prior to contacting with the first substrate composition (column 15, lines 48-51) or after the first detection step (column 13, lines 9-13).

Akhavan-Tafti '979 teaches first and second enzyme conjugates that are bound directly to probes or are bound to first and second target molecules that are bound to probes (columns 15-16, Example 2; column 14, lines 29-33 and 60-67; column 4, lines 31-40; column 5, lines 18-24). Also disclosed are antidigoxigenin:enzyme conjugates wherein the corresponding target molecules are labeled with digoxigenin ((columns 15-16, Example 2). The enzyme conjugates are added prior to the addition of the substrate compositions (see column 15, lines 40-45 for example).

Akhavan-Tafti '979 also discloses contacting a support surface with a sample comprising first and second target molecules labeled with a first second label (e.g., lambda phage DNA labeled with biotin and SPPI marker DNA labeled with digoxigenin) prior to contacting the support surface with the substrate composition (columns 15-16, Example 2 in particular). With regard to claim 31, the surface layer may be washed after contact with the first target molecule

and prior to contact with the first chemiluminescent substrate (see column 15, lines 48-51). Target molecules can include pools of target nucleic acids and mRNA for expression studies (column 14, lines 12-17) and may be quantified (column 1, lines 55-58).

Akhavan-Tafti '979 fails to specifically teach a method wherein a plurality of probes is disposed on the surface layer at a density of at least 50 or at least 100 discrete areas per cm^2.

However, Akhavan-Tafti et al. teach chemiluminescent detection of DNA in low- and medium-density arrays of 100 spots per cm^2 (p. 2065, right column, paragraph 4). Akhavan-Tafti further teach that such arrays are useful in high-throughput analysis of gene mutations and gene expression (p. 2065, right column, paragraph 1) and can be combined with chemiluminescent analysis with no expensive instrumentation (p. 2066, right column, last paragraph).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the plurality of probes disposed on a surface layer at a density of at least 50 or 100 discrete areas per cm^2 as taught by Akhavan-Tafti et al. in the method of detecting chemiluminescent emissions of Akhavan-Tafti '979, because Akhavan-Tafti et al. teaches the benefit of arrays in allowing for high-throughput analysis in methods for chemiluminescent detection of biological molecules, such as that of Akhavan-Tafti '979. One would have had reasonable expectation of success in combining the array of Akhavan-Tafti et al. with the sequential chemiluminescent detection method of Akhavan-Tafti '979 because Akhavan-Tafti et al. established that chemiluminescent detection was feasible with array formats.

16. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti (US Patent No. 6,068,979) as applied to claims 2, 5, 28, and 44 above, or,

alternatively, over Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Wang et al. (WO 01/73134 A2).

As discussed above, Akhavan-Tafti '979 teaches binding pairs such as digoxigenin-antidigoxigenin in methods of determining chemiluminescent emissions on a solid support. Akhavan-Tafti '979 also teaches sequential chemiluminescent detection for measuring levels of target species such as mRNA (column 6, lines 25-34). However, Cheek et al. and Akhavan-Tafti '979 et al. fail to teach a method wherein cDNA target molecules are labeled with digoxigenin.

Wang et al. teach ordered arrays of pools of target molecules (nucleic acids) on a solid support, where the mixtures reflect the expression profile of different cells or tissues (the abstract). Target molecules include cDNA (p. 24, lines 28-32 and p. 26, lines 24-33), which can be used in microarray methods for analysis of gene expression in place of mRNA target samples because cDNA is more stable.

It would have been further obvious to one of ordinary skill in the art to employ cDNA target molecules as taught by Wang et al. in the method of sequential chemiluminescent detection using digoxigenin-antidigoxigenin binding of Cheek et al. and Akhavan-Tafti '979 when detecting mRNA target species, because Wang teaches that cDNA target molecules derived from mRNA are more stable. One would have reasonable expectation of success because Wang further teaches that cDNA target molecules may be effectively used in array formats, such as that of Cheek et al. and Akhavan-Tafti '979.

17. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Huang ("Detection of multiple proteins in an antibody-based protein microarray system," *Journal of Immunological Methods* **255**:1-13 (2001)).

Cheek et al. fail to teach a method wherein the first and second enzyme conjugates are each bound directly to probes; that is, where the target molecules in the sample are directly labeled with an enzyme (see the specification, p. 7, lines 22-24).

Huang teaches detection of multiple proteins in a protein microarray using enhanced chemiluminescence detection. In one embodiment, immunoglobulin probes were spotted onto a membrane and detected by incubation with antibody targets that were conjugated with HRP (see p. 4-6, section 3.1 and Table 2). Huang teaches that detection of the HRP targets binding to immobilized immunoglobulins was useful in testing the specificity and sensitivity of the assay (p. 6, left column, paragraphs 2-4).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ target molecules directly labeled with an enzyme, as taught by Huang et al., in order to assess the specificity and sensitivity of an assay involving a protein microarray with chemiluminescent detection, such as that of Cheek et al.

18. Claims 8 and 40-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti (US Patent No. 6,068,979) as applied to claims 2, 5, 28, and 44 above, and further in view of Bronstein et al. (US Patent No. 6,602,658 B1).

Cheek et al. and Akhavan-Tafti '979 are as discussed above, which fail to teach a method wherein the surface layer is contacted with quantum yield enhancing material before contacting the surface layer with the composition comprising the first chemiluminescent substrate, or where the quantum yield enhancing material is an onium polymer selected from the group listed in claim 40, an onium copolymer, or where the composition comprising the quantum yield enhancing material further comprises an additive selected from the group listed in claim 42.

Bronstein et al. teach a method of measuring gene activity using sequential chemiluminescent detection of signal from two or more chemiluminescent substrates, wherein chemiluminescent signal enhancers such as polyvinylbenzyltrimethylammonium chloride (TMQ), onium copolymers, or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide the quantum yield enhancing material before contacting the surface layer with the first chemiluminescent substrate composition because Bronstein et al. teaches that the enhancer may be successfully added at any point in a methods for chemiluminescent detection of multiple substrates, such as those of Cheek et al. and Akhavan-Tafti '979. It would have been further obvious to include BSA as an additive because Bronstein et al. teach that both BSA and onium polymers serve to enhance the chemiluminescent signal. In addition, it would have been obvious to employ polyvinylbenzyltrimethylammonium chloride or an onium copolymer because Bronstein et al. teach that these are enhancers capable of significantly increasing the intensity of the chemiluminescent signal emitted in chemiluminescent detection methods.

19. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti et al. (*Clinical Chemistry* **44**:2065-2066 (1998)).

Cheek et al. fail to teach a method wherein the density of discrete areas on the surface layer is at least 100 discrete areas per cm^2.

As discussed above, Akhavan-Tafti et al. teach chemiluminescent detection of DNA in low- and medium-density arrays of 100 spots per cm^2 (p. 2065, right column, paragraph 4). Akhavan-Tafti et al. further teach that such arrays are useful in high-throughput analysis of gene mutations and gene expression (p. 2065, right column, paragraph 1) and can be combined with chemiluminescent analysis with no expensive instrumentation (p. 2066, right column, last paragraph).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the plurality of probes disposed on a surface layer at a density of at least 50 or 100 discrete areas per cm^2 as taught by Akhavan-Tafti et al. in the method of detecting chemiluminescent emissions of Cheek et al., because Akhavan-Tafti et al. teaches the benefit of such higher density arrays in allowing for high-throughput analysis in methods for chemiluminescent detection of biological molecules, such as that of Cheek et al.

20. Claims 16-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Wang et al. (WO 01/73134 A2).

Akhavan-Tafti '979, Akhavan-Tafti et al., and Cheek et al. are as discussed above, which fail to teach methods wherein the target molecules are pools of nucleic acids, comprise mRNA transcripts, cDNA or cRNA transcripts, or wherein the concentration of target nucleic acids is proportional to the expression level of genes.

Wang et al. teach ordered arrays of pools of target molecules (nucleic acids) on a solid support, where the mixtures reflect the expression profile of different cells or tissues (the abstract). Detection by nucleic acid hybridization using labeled probes may be through

chemiluminescence (p. 3, line 35 to p. 4, line 2). Wang et al. further teach that target molecules may comprise mRNA or may be DNA derived from mRNA (such as cDNA) in order to provide a relatively accurate indication of the level of expression of each gene in a cell (see p. 24, lines 21-37 and p. 26, lines 24-28 in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ the chemiluminescence detection method of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. to detect pools of nucleic acids such as mRNA or cDNA as taught by Wang et al. because Wang et al. teach that such mRNA or mRNA-derived target molecules indicate of the level of expression of each gene in a cell, making them useful for gene expression studies. One would have reasonable expectation of success in using these target molecules in the methods of Cheek et al. and Akhavan-Tafti '979/Akhavan-Tafti et al. because Wang et al. teach that chemiluminescent detection may be employed in nucleic acid hybridization detection methods.

21. Claims 22-23 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. or, alternatively, Akhavan-Tafti '979 view Akhavan-Tafti et al., in further in view of Ferea et al. (US Patent 6,905,826 B2). Cheek et al., Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein control probes are located on the support surface, or wherein control probes are co-located in one or more of the same discrete areas as the analyte probes.

Ferea et al. teach methods for detecting of target molecules in a sample using nucleic acid microarrays and in particular controls signals to be used in such methods. Such control signals allow for correction of irregularities in the shape, size, and intensity of microarray features (column 5, lines 49-52). Control signals additionally may be used to quantify the experimental

signal (column 6, lines 16-19). Control oligonucleotide probes deposited on the array in the same discrete areas ("features") as the experimental probes can be used as hybridization controls (see column 6, lines 41-60; claim 1 and Figure 4 in particular). Control labels may include fluorescent labels, to be used in conjunction with chemiluminescence labeling of experimental target molecules (see claim 17 in particular).

Ferea et al. further teach introduction of control labels, which may include fluorescent labels, to the surface of the array, which are used to calculate the relative amount of multiple experimental target sequences by comparing the ratios of the intensity of the experimental and control label signals (column 6, lines 15-19 and claim 15).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the control probes taught by Ferea et al. in the method of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. because Ferea et al. teach the benefit of control probes in determining whether hybridization is occurring in a microarray-format nucleic acid hybridization method.

It would have been further obvious to include in the method of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. a fluorescent label as a control label and to compare the intensity of the signal from the fluorescent label to the experimental chemiluminescent signals because Ferea et al. teach that such control labels may be used to help quantify experimental signals in a microarray-format nucleic acid hybridization method using chemiluminescent and/or fluorescent detection.

22. Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Yang et al. (WO 01/83814 A2). Cheek et al. fail to teach a method wherein the support surface further comprises fluorescent labels.

Yang et al. teach a method for analysis of gene expression, wherein probes are immobilized on a solid support (bead) containing a fluorochrome (see the abstract and p. 15, lines 4-9 and 14-15 and p. 17, lines 12-23). Detection of hybridization of a target nucleic acid molecule to the immobilized probe may be through chemiluminescence (p. 3, lines 4-5 and p. 17, lines 3-11). The fluorochrome labels are attached directly to the solid support in that the beads are stained with the fluorochrome (p. 14, lines 8-24).

23. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Gambini et al. (US Patent No. 6,518,068 B1). Cheek et al., Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein detection of the second chemiluminescent signal comprises filtering the emissions with a filter adapted to reduce the intensity of the first chemiluminescent signal relative to the intensity of the second.

Gambini et al. teach a detection workstation for analysis of luminescent signals that comprises a filter (or filters on a filter wheel) which permits the selection of different wavelength ranges, and which may be used to separate the emissions of different reagents emitting at different wavelengths. The workstation may be used in a method for detecting multiple luminescent signals emitting at different wavelengths (see the abstract and column 6, line 55 to column 7, line 13). Gambini et al. further teach that signals from multiple reagents are separated using the filters, which are designed to maximize the sensitivity of the target reagent emission,

while minimizing the sensitivity to other non-target reagent emission (column 13, line 60 to column 14, line 10).

Therefore, it would have been obvious to one of ordinary skill in the art to employ the filter detection method taught by Gambini et al. in the methods of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. because Gambini et al. teach that such filters may be used to separate signals at different wavelengths by multiple reagents in a method for detection of multiple luminescent signals.

24. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Bronstein et al. (US Patent No. 4,931,223). Cheek et al. fail to teach a method wherein the two enzymes are beta-galactosidase and alkaline phosphatase.

Bronstein et al. teach methods of chemiluminescent detection for detection of multiple analytes in a sample, employing two enzyme conjugates that are beta-galactosidase and alkaline phosphatase conjugates (column 8, lines 1-21).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to substitute beta-galactosidase as taught by Bronstein et al. for the horseradish peroxidase enzyme used by Cheek et al. because Bronstein et al. teach that beta-galactosidase is also a suitable enzyme for use in conjunction with alkaline phosphates in a two-enzyme chemiluminescence method for detection of multiple analytes in a sample, such as that of Cheek et al.

25. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Bronstein et al. as applied to claim 34 above, or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and

44, and further in view of Voyta et al. (US Patent No. 5,145,772). Cheek et al. and Bronstein et al. fail to teach a method wherein the composition comprising a chemiluminescent substrate capable of being activated by alkaline phosphatase is a 0.1M solution of aminomethylpropanol and 1 mM $MgCl_2$ at a pH of 9.5.

Voyta et al. teach a chemiluminescence detection method wherein alkaline phosphatase is used in a solution containing 0.05M carbonate or Tris buffer solution and 1 mM $MgCl_2$ at pH=9.5 (column 11, lines 49-55 and column 13, lines 10-17). Although Voyta et al. teach carbonate rather than sodium phosphate, it is well known in the art that these buffers may be interchanged in order to maintain a solution pH of 9.5. Further, while the concentration of the buffer used by Voyta et al. differs, such a difference will generally not support patentability in most cases, constituting optimization of ranges within prior art conditions (see MPEP 2144.05).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a solution comprising a buffer with a buffering capacity sufficient to maintain a pH of 9.5 in conjunction with 1 mM $MgCl_2$ in the method of Cheek et al. and Bronstein et al. because Voyta teaches that such a solution is effective in a chemiluminescence detection method employing alkaline phosphatase.

26. Claim 36 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view Bronstein et al., or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Bobrow et al. (US Patent No. 5,196,306). Cheek et al. and Bronstein et al. fail to teach a method wherein the composition comprising a chemiluminescent substrate capable of being activated by beta-galactosidase is a 0.1M solution of sodium phosphate and 1 mM $MgCl_2$ at a pH of 7.0.

Bobrow et al. teach use of beta-galactosidase in a solution comprising of 10 mM sodium phosphate, 1 mM $MgCl_2$ at pH 7.0 (column 17, paragraph 2). Beta-galactosidase stored in this solution was shown to be active (Figure 7).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a substrate solution containing 0.1M solution of sodium phosphate and 1 mM $MgCl_2$ at a pH of 7.0 because Bobrow et al. teach that a solution comprising sodium phosphate buffer and 1 mM $MgCl_2$ was an appropriate solution that would not destroy enzyme activity in an assay involving beta-galactosidase, such as that of Cheek et al. and Bronstein et al.

27. Claims 37-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and further in view of Clothier (US Patent No. 6,852,503 B1). Akhavan-Tafti '979 in view of Akhavan-Tafti et al. fail to specifically recite contacting a support surface with a composition comprising the first and second enzyme conjugates. However, Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Clothier teaches a dual enzyme chemiluminescent substrate formulation for use in methods involving two enzymes. Clothier teaches combining the two chemiluminescent enzymes

(horseradish peroxidase and alkaline phosphatase) together prior to contacting the enzymes with the well surface (column 6, lines 25-36).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the enzyme conjugates of Akhavan-Tafti '979 prior to contacting with the support surface since Clothier teaches that enzymes for chemiluminescent substrates may be successfully combined together in methods for chemiluminescence detection involving two enzymes, such as that of Akhavan-Tafti '979. It would have been further obvious to employ this step taught by Clothier in a method wherein the first and second enzyme conjugates that comprise enzyme-antibody conjugates and wherein the first and second target molecules are labeled with an antigen for the antibody because antibody-enzyme conjugates are taught by Akhavan-Tafti '979.

28. Claim 37 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. as applied to claims 1, 3-4, 13-15, 21, 29 and 31-32 above, and further in view of Clothier (US Patent No. 6,852,503 B1). Cheek et al. fail to specifically recite contacting a support surface with a composition comprising the first and second enzyme conjugates.

Clothier teaches a dual enzyme chemiluminescent substrate formulation for use in methods involving two enzymes. Clothier teaches combining the two chemiluminescent enzymes (horseradish peroxidase and alkaline phosphatase) together prior to contacting the enzymes with the well surface (column 6, lines 25-36).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the enzyme conjugates of Cheek et al. and Akhavan-Tafti '979 prior to contacting with the support surface since Clothier teaches that enzymes for chemiluminescent substrates may be successfully

combined together in methods for chemiluminescence detection involving two enzymes, such as that of Cheek et al. and Akhavan-Tafti '979.

29. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Clothier as applied to claim 37 above, and further in view of Akhavan-Tafti '979. Cheek et al. and Clothier et al. fail to teach a method wherein both the first and second enzyme conjugates that comprise enzyme-antibody conjugates and wherein the first and second target molecules are labeled with an antigen for the antibody

Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Therefore, it would also have been obvious to one of ordinary skill in the art at the time of the invention to substitute the streptavidin-HRP conjugate of Cheek et al. with an antidigoxigenin-HRP conjugate for detection of a second target molecule labeled with digoxigenin, because Akhavan-Tafti '979 teaches that antigen-antibody and biotin-streptavidin are both examples of binding pairs that may be successfully used in a method of sequential chemiluminescent detection of multiple, differentially labeled target molecules such as that of Cheek et al.

30. Claim 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and further in view of Greene et al. (US Patent No. 5,137,804).

Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to teach a method wherein the first substrate is a 1,2-dioxetane substrate and the second is an acridan, enol phosphate, or luminol substrate. Akhavan-Tafti '979 teaches that the first chemiluminescent substrate used in a sequential detection method must be capable of being inhibited, and that the use of a horseradish peroxidase substrate such as an acridan compound is preferred as the first chemiluminescent substrate because of the ability to inhibit peroxidase activity (column 7, lines 65-67 to column 8, lines 1-23).

Greene et al. teach inhibitors of the enzyme alkaline phosphatase, which include inorganic phosphate, chelating agents, and amino acids (column 6, lines 9-29), in the context of enzyme-based detection methods using alkaline phosphatase, horseradish peroxidase, and other enzymes (column 6, lines 30-37 and column 10, lines 19-30).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a horseradish peroxidase substrate (such as an acridan) in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. as the second, rather than the substrate, and to use a 1,2-dioxetane substrate capable of being activated by alkaline phosphatase as the first, rather than the second substrate, because Greene et al. teaches that alkaline phosphatase may also be readily inhibited in assays that employ this enzyme, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

31. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 as applied to claims 2, 5, 28, and 44 above, and further in view of Akhavan-Tafti et al. (US Patent No. 5,523,212). Cheek et al. and Akhavan-Tafti '979 are as discussed above, which fails to specifically teach a composition comprising a chemiluminescent quantum yield enhancing material that further comprises counterion moieties listed in claim 43.

Akhavan-Tafti '212 teach chemiluminescent formulations for the detection of biological molecules that comprise enhancers and additives such as β-cyclodextrin, polyols, and sulfate (column 15, lines 45-59; column 16, lines 1-21; and Examples 13 and 20), and it is further taught that useful levels of light intensity compared to reagent background are obtained with reagents that incorporate dextran sulfate and β-cyclodextrin (Example 20).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ dextran sulfate and β-cyclodextrin, as taught by Akhavan-Tafti '212 in the chemiluminescent formulations comprising enhancers of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '212 teaches that such additives give rise to useful levels of light intensity in methods for chemiluminescent detection of biological molecules, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

32. Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. (*Clinical Chemistry* **44**:2065-2066 (1998)) as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and as evidenced by Girotti et al. and Akhavan-Tafti (Girotti et al., "Chemiluminescent Immunoperoxidase Assay for the Dot Blot Hybridization Detection of Parvovirus B19 DNA

Using a Low Light Imaging Device," *Analytical Biochemistry* 236:290-295 (1996) and US Patent No. 5,650,099).

Use of the chemiluminescent substrates Lumigen PPD and PS-3, which have different emission maxima of 470 nm and 430 nm, respectively, as evidenced by Akhavan-Tafti (US Patent No. 5,650,099, column 22, lines 15-26) and Girotti et al. (p. 290, right column, paragraph 3) is taught by Akhavan-Tafti '979 (column 9, lines 46-47; column 10, lines 42-45, and column 15, lines 1-8).

33. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. (*Clinical Chemistry* **44**:2065-2066 (1998)) and as evidenced by Girotti et al. and Akhavan-Tafti as applied to claim 27 above, and further in view of Akhavan-Tafti et al. (US Patent No. 6,045,727).

Akhavan-Tafti et al. (US Patent No. 6,045,727) teach substrates for chemiluminescent detection. In particular, Akhavan-Tafti '727 teaches an Acridan Derivative 1, which has an emission maxima of 430 nm and is capable of being activate by alkaline phosphatase (Examples 1, 14, and 19).

Therefore, it would have been obvious to one of ordinary skill in the art to substitute the Lumigen PPD substrate of Akhavan-Tafti '979 with the Acridan Derivative 1 in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '727 teaches that Acridan Derivative 1 is a substrate for a hydrolytic enzyme, which is a requirement for the second enzyme in the sequential chemiluminescent detection method of Akhavan-Tafti '979 and Akhavan-Tafti et al.

34. Claims 42-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and further in view of Akhavan-Tafti (US Patent No. 5,523,212).

Akhavan-Tafti '979 and Akhavan-Tafti et al. teach preferred formulations for chemiluminescence but fails to specifically teach additives and counterions. Akhavan-Tafti '212 teach chemiluminescent formulations for the detection of biological molecules that comprise enhancers and additives such as β–cyclodextrin, polyols, and sulfate (column 15, lines 45-59; column 16, lines 1-21; and Examples 13 and 20), and it is further taught that useful levels of light intensity compared to reagent background are obtained with reagents that incorporate dextran sulfate and β–cyclodextrin (Example 20).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ dextran sulfate and β-cyclodextrin, as taught by Akhavan-Tafti '212 in the chemiluminescent formulations comprising enhancers of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '212 teaches that such additives give rise to useful levels of light intensity in methods for chemiluminescent detection of biological molecules, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

35. Claims 6 and 33-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and further in view of Bronstein et al. (US Patent No. 4,931,223). Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to specifically teach a method wherein both the first and second chemiluminescent substrates are 1,2-dioxetane substrates or a method where the enzymes are β-galactosidase and alkaline phosphatase.

Bronstein et al. teach methods for detecting chemiluminescent emissions using two or more 1,2-dioxetane substrates, which may be used in quantifying several analytes when each of the 1,2-dioxetanes emits light of a different wavelength (column 2, line 42 to column 3, line 3; 6, lines 44-47; column 7, lines 5-19; column 8, lines 1-29 in particular). Bronstein et al. further teach that β-galactosidase and alkaline phosphatase may be used in the method for cleaving different cleavable dioxetane substituents, and that use of these enzymes to cleave 1,2-dioxetane substrates that emit light of different wavelengths enables multichannel assays to be performed (column 7, lines 5-19; column 13, "Assay Procedure").

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ two 1,2-dioxetane substrates in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. because Bronstein et al. teach that two or more 1,2-dioxetane substrates may be successfully used in a chemiluminescent detection assay for the quantification of two or more analytes, such as that of Akhavan-Tafti and Akhavan-Tafti et al., in order to enable multichannel assays.

36. Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and further in view of Bronstein et al. (US Patent No. 6,602,658 B1).

Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein the surface layer is contacted with the enhancing material prior to contacting with the first chemiluminescent substrate composition.

Bronstein et al. teach a method of measuring gene activity using sequential chemiluminescent detection of signal from two or more chemiluminescent substrates, wherein

chemiluminescent signal enhancers such as onium copolymers, polyvinylbenzyltrimethylammonium chloride or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide the quantum yield enhancing material before contacting the surface layer with the first chemiluminescent substrate composition, because Bronstein et al. teach that the enhancing material may be added at any point in a method of sequential detection of chemiluminescent signals such as that of Akhavan-Tafti '979.

37. Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 above, and further in view of Akhavan-Tafti et al. (US Patent 5,843,666). Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to teach a chemiluminescent detection method in which the support surface further comprises fluorescent labels.

Akhavan-Tafti '666 teaches chemiluminescent detection methods for detection of multiple DNA sequences, wherein DNA is immobilized on a solid support (nylon membrane). The DNA comprises a fluorescent label (fluorescein), which enables chemiluminescent detection following recognition by an anti-fluorescein antibody-enzyme conjugate (HRP-anti-fluorescein) (columns 12-13, Example 1). The fluorescent label is therefore attached to the surface layer via the immobilized DNA.

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the fluorescent label of Akhavan-Tafti '666 in the method of Akhavan-Tafti and Akhavan-Tafti et al. for the purpose of enabling recognition by an anti-fluorescein antibody-enzyme conjugate and allowing for signal detection in a method of chemiluminescent detection.

Response to Arguments

The provisional rejection of claims 1-45 under the judicially created doctrine of obvious-type double patenting as being unpatentable over US Application Nos. 10/620,333 and 10/462,742 is withdrawn in light of Applicant's submission of a terminal disclaimer on November 1, 2005, which was approved by the Office on 1/4/06.

The rejections of claims 5, 8, 13, 15, 17-18, 21-26, 30, and 35-38 under 35 USC 112, 2nd paragraph are withdrawn in response to Applicant's amendments. However, the claims remain rejected due to their dependence on independent claim 1.

The rejection of claim 1 under 35 USC 112, 2nd paragraph is maintained (see the previous Office action at p. 9 and the rejection above). Applicant has amended the claim to recite that the probes are bound to the enzyme conjugates prior to contacting the surface layer with the substrate compositions, but it remains unclear whether the description of binding between probes and enzyme conjugates refers to an active process step that occurs during the performance of the method, or a description of structural attachment of these molecules that is constant throughout the method.

The rejection of claims 3-4 and 7 is maintained for similar reasons (see above and the previous Office action at p. 9, item 11.

Applicant's arguments are persuasive to overcome the rejection of claim 25 under 35 USC 112, 2nd paragraph.

Applicant's amendments to claim 31 have presented new grounds of rejection under 35 USC 112, 2nd paragraph (see above).

Regarding the rejection of claims 10-12 under 35 USC 112, first paragraph, Applicant's arguments filed 11/1/05 have been fully considered but they are not persuasive. Applicant argues that the specification provides an enabling disclosure for at least the subject matter of claim 10, in that the specification incorporates by reference International Publication No. WO 99/53319. This is not persuasive because the incorporation of essential material in the specification by reference to an unpublished U.S. application, foreign application or patent, or to a publication is improper. Applicant is required to amend the disclosure to include the material incorporated by reference, if the material is relied upon to overcome any objection, rejection, or other requirement imposed by the Office. The amendment must be accompanied by a statement executed by the applicant, or a practitioner representing the applicant, stating that the material being inserted is the material previously incorporated by reference and that the amendment contains no new matter. 37 CFR 1.57(f).

In addition, the subject matter of WO 99/53319 is not commensurate with the scope of the claims at issue. As noted in the previous office action on p.4, "[t]he claims are not limited to arrays produced by any particular method and are not limited to a type of probe biomolecule that is immobilized on the support." The claims are not limited to a support surface comprising undulations resulting from relaxation of the solid support as in WO 99/53319 (see the

specification at p. 14, lines 11-17). The claims are also not limited to a type of solid support material, but the solid support of WO 99/53319 must be a polymeric material (see p. 6-7 of the previous Office action and p. 7-10 of WO 99/53319).

Furthermore, the Examiner maintains that the bulk of the prior art teaches that microarrays of the claimed feature densities were **not** well known in the art at the time of the invention, particularly in the case of protein-based microarrays (see p. 4-8 of the previous Office action). It is noted that MPEP 2164.03 teaches that "the amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability of the art. In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The amount of guidance or direction refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention. The more that is known in the prior art about the nature of the invention, how to make, and how to use the invention, and the more predictable the art is, the less information needs to explicitly stated in the specification. In contrast, if little is known in the prior art about the nature of the invention and the art is unpredictable, the specification would need more detail as how to make and use the invention in order to be enabling."

For the above reasons and in light of the unpredictability associated with chemiluminescent signal detection (see the previous Office action at p. 7), it is maintained that the specification fails to teach the skilled artisan how to make and use the invention of claims 10-12 without undue experimentation.

Regarding the rejections of claims 1, 3-4, 13-15, 21, 29, and 31-32 under 35 USC 102 as being anticipated by Cheek et al., and of claims 25 and 27 under 35 USC 102 as being anticipated by Cheek as evidenced by Weimer et al., Applicant argues that Cheek et al. does not disclose a plurality of probes disposed in a plurality of discrete areas on a surface layer of a solid support. This argument has been fully considered but is not persuasive. Cheek discloses a solid support with a surface layer upon which a plurality of discrete areas (microchannels) is located, and wherein a plurality of probes is located in the plurality of discrete areas. This argument is not found persuasive, as the claims do not require that the discrete areas be two-dimensional. To the contrary, the specification discloses that the support surface may be two-dimensional or non-planar (p. 14, lines 11-17). Applicant further argues that in Cheek, the probes are not located on the surface layer but rather are within the volumes formed by the microchannels of the solid support, which are internal to the solid support. This argument is not persuasive because the claims do not recite, and the specification does not indicate, that the probes are not internal to the solid support.

With regard to the rejection of claims 2, 5, 7-9, 16-19, 22-24, 26, 28, 30, 34-38, and 40-45 under 35 USC 103 as being unpatentable over Cheek et al., Applicant argues again that Cheek et al. does not anticipate the method of claim 1 for the reasons discussed above, but has not set forth any additional arguments as to why the rejections of record should be withdrawn, (see pages 16 and 18-25 of Applicant's amendment). Therefore, the rejections are maintained because the arguments with regard to claim 1 have not been found persuasive.

Regarding the rejection of claims 1-5, 7, 9, 13-15, 21, 28-29, 31-32, 40-41, and 44 under 35 USC 103(a) as being unpatentable over Akhavan-Tafti '979 in view of Akhavan-Tafti et al. ("Akhavan-Tafti"), Applicant argues that Akhavan-Tafti is directed to the use of a specific alkaline phosphatase substrate, and that this specific substrate, Lumigen APS was key to the success of the method, such that one of ordinary skill in the art would not have been motivated to combine Akhavan-Tafti with Akhavan-Tafti '979, since Akhavan-Tafti '979 involves the use of a chemiluminescent peroxidase substrate and not an alkaline phosphatase substrate (see Applicant's response, p. 17-18). This argument is not found persuasive, because while the method of Akhavan-Tafti '979 involves the use of a peroxidase substrate as the first chemiluminescent substrate, the second chemiluminescent substrate used in the method is a substrate for a second enzyme that is "preferably a hydrolytic enzyme, preferably selected from alkaline phosphatase, β-galactosidase and glucuronidase, *more preferably alkaline phosphatase*" (Akhavan-Tafti '979, column 8, lines 53-56, emphasis added). Therefore, the Examiner disagrees that the use of a peroxidase substrate in Akhavan-Tafti '979 would lead away from the claimed invention, since Akhavan-Tafti '979 also teaches the use of alkaline phosphatase substrates, as in Akhavan-Tafti.

With regard to the argument that the specific alkaline phosphatase substrate used in Akhavan-Tafti was the "key to success" as indicated by the passage therein on p. 2066, the last sentence, this argument is not found persuasive. The passage appears to be in the context of describing the properties of the Lumigen APS substrate, namely, the ability to permit rapid detection at room temperature (see p. 2066, the last paragraph). The Examiner disagrees, as Applicant appears to suggest, that the reference teaches away from the use of the any other

substrate, as Akhavan-Tafti does not teach that Lumigen APS-5 would be the only substrate compatible with such a method. One of ordinary skill in the art would conclude from the above passage that the rapid chemiluminescent detection method of Akhavan-Tafti for analyzing DNA arrays on nylon membranes or coated glass slides would require a substrate with the above-mentioned characteristics, so as to permit rapid detection at room temperature.

Regarding the rejection of claims 16-19, 45 under 35 USC 103(a) as being unpatentable over Akhavan-Tafti '979, Applicant argues again that the combination of Akhavan-Tafti '979 and Akhavan-Tafti does not render the method of claim 1 unpatentable for the reasons discussed above, but has not set forth any additional arguments as to why the rejections of record should be withdrawn, (see pages 20-28 of Applicant's amendment). Therefore, the rejections are maintained because the arguments with regard to claim 1 are not persuasive.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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